09/142,660

Appendix 1





BIOSENSORS 98

The Fifth World Congress on Biosensors

Inter-Continental Hotel, Berlin, Germany
3-5 June 1998

REFEREED ABSTRACTS

ELSEVIER SCIENCE

paper at 5th World Congress on Biosensor June 4, 1998, Berlin

Nanometer scaled DIT used for lable free DNA analysis

M.Paeschke*, R.Seitz, L.M.Buchmann, R.Hintsche
Fraunhofer Institut für Siliziumtechnologie, Fraunhoferstr.1, D-25524 Itzehoe
Tel. 0049 48214306, Fax: 0049 48214350, e-mail: paeschke@isit.fhg.de

Keywords: *nanoscaled interdigitated electrodes, lable free detection, DNA-analysis, impedance spectroscopy*

The microfabrication of DNA analytical systems show growing evidence in terms of costs materials and performances. Such systems are manly based on optical and electrical detection methods.

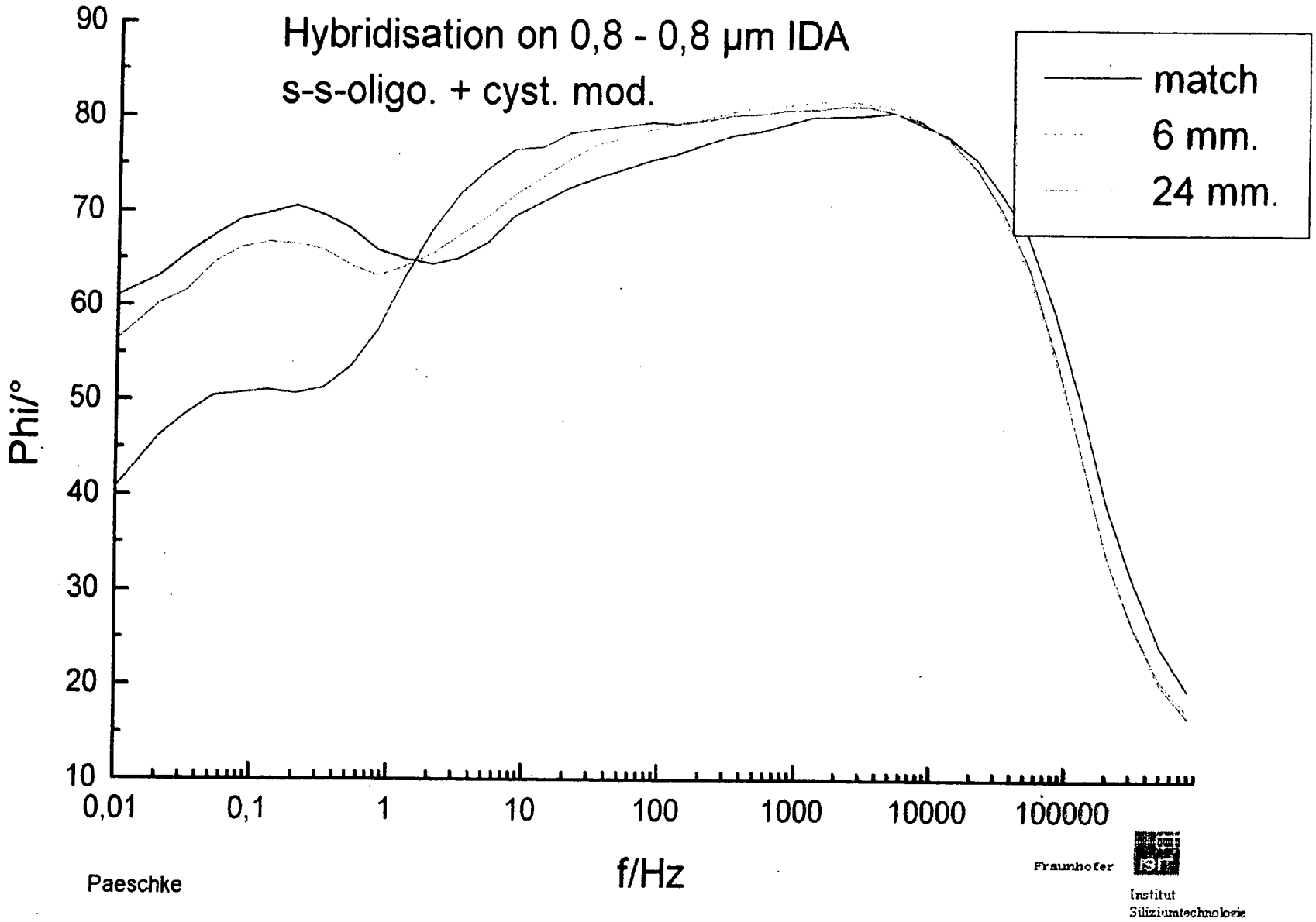
We are currently developing and manufacturing electrochemical transducers in nanometer dimensions in order to perform a lable free detection of nucleic acids. First results with nanometer scaled and microfabricated Dielectric Interdigitated Transducers (DIT) are previously described [1]. The measuring concept is based on capacitance and admittance measurements on two interdigitated electrode bands, where the dielectric properties of the electrode surfaces and their double layers are changed by deposition of complexing nucleic acid molecules onto the electrode surface.

The DIT measurement allows as well the observation of the binding of oligonucleotides onto the transducer surface and as the detection of hybridized DNA. The deposition of oligonucletides (24mer) were realized by self assembling of the gold electrode of their disulfide derivates and alternatively over covalent coupling via Si-OH groups at the electrode gaps. The decrease of the DIT-capacitance is shown for the electrode surface loading, whereas the admittance spectroscopy indicate the gap modification. The DNA hybridization have been observed in dependence of different surface concentrations of oligonucleotides and with oligonucleotides with missmatches. 6 and 24 mismatches of oligonucleotides were used. The capacitance and admittance changes are shown the hybridization dependence of the number of missmatches. The results will be compared with fluorescence experiments.

The novel analytical principle of microfabricated DIT is a powerful tool for the DNA analysis and further integration in analytical microsystems.

[1] M.Paeschke, R.Seitz, U.Schnakenberg, R.Hintsche; BIOSENSORS 96, Bangkok, 72, 199
M.Paeschke, R.Seitz, L.M.Buchmann, R.Hintsche;
Micro System Technologies 96 (ed. H.Reichl, A.Heuberger) VDE-Verlag, 369, 1996

refereed abstracts p. 110


Hybridisation on 0,8 - 0,8 µm IDA
s-s-oligo. + cyst. mod.
match
6 mm.
24 mm.
Phi/°
90
80
70
60
50
40
30
20
10
0,01
0,1
1
10
100
1000
10000
100000
f/Hz
Paeschke
Fraunhofer
Institut
Siliziumtechnologie

Sequenzen ad Plasmid-Hybridisierung an Au-S immob. Fänger-Oligos auf IDA für Impedanzmessungen

Oligo 1:

Sequenz: 5' CGC CAG GGT TTT CCC AGT CAC GAC 3'-SS-X

Oligo 2:

Sequenz: 5' CGC CAG GGT **CCC TTT AGT CAC GAC** 3'-SS-X

Oligo 3:

Sequenz: 5' **TTG ACA CCA GAC CAA CTG GTA ATG**3'-SS-X

korrespondierende Plasmid-Sequenz:

.....5' GTC GTG ACT GGG AAA ACC CTG GCG 3'.....

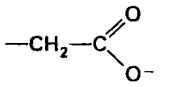
REVERSIBLE INTERACTIONS OF BIOMOLECULES ARE MEDIATED BY THREE KINDS OF NONCOVALENT BONDS

Reversible molecular interactions are at the heart of the dance of life. Weak, noncovalent forces play key roles in the faithful replication of DNA, the folding of proteins into intricate three-dimensional forms, the specific recognition of substrates by enzymes, and the detection of signal molecules. Indeed, all biological structures and processes depend on the interplay of noncovalent interactions as well as covalent ones. The three fundamental noncovalent bonds are *electrostatic bonds, hydrogen bonds,* and *van der Waals bonds.* They differ in geometry, strength, and specificity. Furthermore, these bonds are profoundly affected in different ways by the presence of water. Let us consider the characteristics of each:

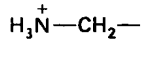
1. *Electrostatic bonds.* A charged group on a substrate can attract an oppositely charged group on an enzyme. The force of such an *electrostatic attraction* is given by Coulomb's law:

$$F = \frac{q_1 q_2}{r^2 D}$$

in which q_1 and q_2 are the charges of the two groups, r is the distance between them, and D is the dielectric constant of the medium. The attraction is strongest in a vacuum (where D is 1) and is weakest in a medium such as water (where D is 80). This kind of attraction is also called an ionic bond, salt linkage, salt bridge, or ion pair. The distance between oppositely charged groups in an optimal electrostatic attraction is 2.8 Å.

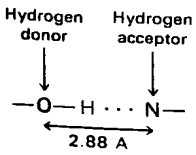


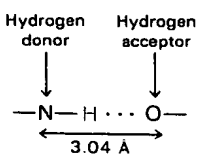
Negatively charged group of a substrate



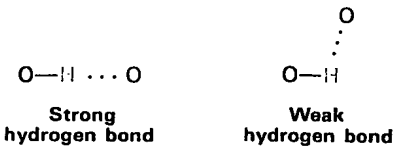
Positively charged group of an enzyme

2. *Hydrogen bonds* can be formed between uncharged molecules as well as charged ones. In a hydrogen bond, *a hydrogen atom is shared by two other atoms.* The atom to which the hydrogen is more tightly linked is called the hydrogen donor, whereas the other atom is the hydrogen acceptor. The acceptor has a partial negative charge that attracts the hydrogen atom. In fact, a hydrogen bond can be considered an intermediate in the transfer of a proton from an acid to a base. It is reminiscent of a ménage à trois.



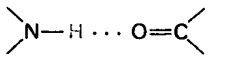


The donor in a hydrogen bond in biological systems is an oxygen or nitrogen atom that has a covalently attached hydrogen atom. The acceptor is either oxygen or nitrogen. The kinds of hydrogen bonds formed and their bond lengths are given in Table 1-1. The bond energies range from about 3 to 7 kcal/mol. Hydrogen bonds are stronger than van der Waals bonds but much weaker than covalent bonds. The length of a hydrogen bond is intermediate between that of a covalent



Strong hydrogen bond

Weak hydrogen bond



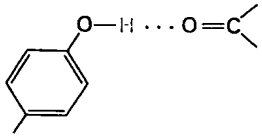


Table 1-1
Typical hydrogen-bond lengths

Bond	*Length* (Å)
O—H···O	2.70
O—H···O⁻	2.63
O—H···N	2.88
N—H···O	3.04
N⁺—H···O	2.93
N—H···N	3.10